

Estimation of Caffeine Concentration in Decaffeinated Coffee and Tea Available in Pakistan

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Tea is an agricultural product of the leaves, tea buds and inter-nodes of the *Camellia sinensis* plant. This study was done to estimate caffeine in different types of decaffeinated tea and coffee brands available in Pakistan's market by High Performance Liquid Chromatography. In this study standard calibration curve of caffeine produced a straight line ($R^2 = 0.999$). . The 0.2% w/v PQC was used and showed retention time of caffeine at 8.665 minutes and IS at 5.337. It was estimated that 0.1807% caffeine present in the positive quality control which provide the mean of the reliability of the performance of the procedure. Total of six brands comprising of 30 decaffeinated samples were selected for the estimation of caffeine. Among all only four (ABS 3, 24, 27 and 28) produced no peak of analyte representing complete decaffeinated product. Possibly, limited sensitivity of instrument may have produced these results but, these are the brands that contain much lesser amount of caffeine as compare to the other brands. Among all 30 decaffeinated samples only (ABS 19) has observed the maximum 0.03% of caffeine and the minimum quantity of caffeine 0.0001% in one sample (ABS 23) was estimated.

Keywords: Caffeine; Coffee; tea; Pakistan; High Performance Liquid Chromatography.

Tea and coffee produces stimulating effect which is because of purine bases that are caffeine, theophylline and theobromine. Other method by which caffeine is obtained is methylation of theobromine. Tea alkaloids are quantified by numerous methods. 3-4% of Caffeine is present in tea which is the major alkaloid (1). Based on the data reviewed, the study showed that coffee produces various effects on health and these effects increase the alertness, change

the mood, hyperactivity, Parkinson's disease, gallstones, diabetes, and also have effect of liver function. Similarly high doses produce adverse effects in some individuals who are sensitive like tachycardia, anxiety and insomnia (2)(3)(4)(5). The healthy individuals may take less than 300-500 mg of caffeine, children should restrict to 50 mg daily and woman should be restricted to 150-200 mg daily. The objective of the study was Estimation of the amount of caffeine in decaffeinated coffee and tea by using HPLC.

Experimental design

Decaffeinated coffee and tea were collected from various super stores to estimate

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the concentration of caffeine. The concentrations of samples were estimated by high performance liquid chromatography done by photo diode array detector (SPD-M20A). Six types of brands were selected for estimation of caffeine. These samples were collected from local market. Samples were collected from capital cities of provinces of Pakistan. Samples were purchased from local market where available. Thirty two samples were collected from each brand in which seven samples for decaffeinated tea and twenty five samples for decaffeinated coffee. Five samples from each brand of decaffeinated coffee were collected and seven samples of decaf tea were collected.

Chemicals

Caffeine Standard (Standpharm Pakistan (Pvt.) Ltd.), Acetaminophen as IS (Standpharm Pakistan (Pvt.) Ltd.), Decaffeinated coffee and tea , Calcium hydroxide (M.W. 74.09, assay 98%, BDH Laboratory Supplies, England), Magnesium Sulphate (M.W 120.37, Assay 98%, Scharlab S.L. Cisa, made in European Union), Dichloromethane HPLC grade (M.W 84.93, Assay 99.8%, Koch-Light Ltd., England), Methanol HPLC/Spectro Grade (M.W 32.0, Assay 99.9%, MP Biomedicals, LLC, France), Acetonitrile HPLC/Spectro Grade (M.W 41.1, Assay 99.9%, MP Biomedicals, LLC, France), Potassium dihydrogen phosphate (98-100.5%, Riedel de haen, RdH Laborchemikalion GmbH & Co.), HPLC grade Water, Distilled water

Instrument

High Performance Liquid Chromatograph was used. Shimadzu HPLC system fitted with autosampler (SIL-20A), reciprocating pump (LC-20AT), solvent delivery system (LC-20A) with system controller (CBM-20A), detection was done by a photo diode array detector (SPD-M20A) at wavelength of 215 nm and Merck C18 column (15 cm x 3.9 mm x 5μ) was used.

Methodology

Preparation of Standard (Stock Solution)

In order to prepare 1000 ppm solution, 1000 mg of caffeine was dissolved in 500 mL of distilled water and in the end make up the final volume 1000 mL in the volumetric flask. Dilutions were prepared from 20,40,60,80,100,120 ppm.

Preparation of Internal Standard (IS)

Acetaminophen was used as IS. It is not present in brands of decaffeinated coffee and tea. Further, it produced reproducible results.

Preparation of Stock IS Solution

In order to prepare 100 mL of 1000 ppm IS solution, 100 mg of IS was dissolved in 50 mL of Methanol and in the end made up the final volume 100 mL in the volumetric flask.

Preparation of Working IS Solution

In order to prepare 100 mL of 500 ppm solution, 50 mL of the stock IS solution was diluted with 30 mL of mobile phase in volumetric flask and, in the end, the final volume made up to 100 mL with mobile phase.

Retention Time Measurement

Retention time of target analyte (Caffeine) and internal standard (Acetaminophen) was determined by running 100 ppm solutions of each, separately, on HPLC.

Preparation of Positive Control

In order to prepare 100 mL of 0.2% w/v positive quality control, 200 mg of caffeine standard was accurately weighed and dissolved in 50 mL distilled water in volumetric flask and, in the end, the final volume made up to 100 mL with distilled water. The solution was refrigerated before use. This was a reference solution or test solution used for assessment of the performance of the procedure.

Preparation of Negative Control

Distilled water was used as negative control sample. It was caffeine free sample. This was a reference solution or test solution used for assessment of the performance of the procedure.

Preparation of Samples

Decaffeinated Tea sample preparation

1.5 gm of decaf tea was dissolved in 200 mL boiling distilled water; it gave light brown color to the solution. This solution was further processed for the estimation of caffeine.

Decaffeinated coffee sample preparation

1.5 gm of decaf Coffee which was dissolved in 200 mL boiling distilled water; it gave blackish brown color to the solution. This solution was further processed for the estimation of caffeine.

Procedure

Wanyika *et al.* 2010 procedure was used with slight changes in this research. 10ml volume of all calibrator, Positive QC, Negative QC and all samples was pipette out in 15 mL centrifuge tubes. 0.8 mL of IS was added to all tubes and vortexed to mix completely. This produced the final strength of 40 ppm of IS. Then 1 gm of calcium hydroxide was

added in all tubes and vortexed. Then the resulting mixture was filter by using whattmann filter paper and then put it into separating funnel. 20 mL of DCM was added and rotated for 15 min to extract caffeine into DCM. DCM was separated from the aqueous phase into 100 mL flat bottom conical flask. This step was repeated two more times and separated DCM layer was added to already separate layer in 100 ml flat bottom conical flask. Then 3 gm of magnesium sulphate was added in each conical flask and mixed thoroughly. The resulting mixtures were filter using whattmann filter paper and the filtrates were dried by evaporation of DCM using rotary evaporator at room temperature. The dried extracts were dissolved in 10 mL of mobile phase and ran on HPLC.

Preparation of Mobile Phase

Altun (2002) used 0.01 M potassium dihydrogen phosphate-methanol-acetonitrile-isopropyl alcohol (84: 4: 6: 6) mobile phase. The

same mobile phase system was used with certain changes. 0.1M potassium dihydrogen phosphate-acetonitrile-methanol (60: 10: 30) was used as mobile phase having pH of 5.4.

Chromatographic Conditions

HPLC

Shimadzu HPLC system fitted with autosampler (SIL-20A), reciprocating pump (LC-20AT) and Merck C18 column (15 cm x 3.9 mm x 5μ) was used for separation. A Shimadzu HPLC solvent delivery system controller (LC-20A) with system controller (CBM-20A) was used. The 20μL sample was used in injection system. Detection was done by photo diode array detector (SPD-M20A) at wavelength of 215 nm (Altun, 2002). Calculation was performed using following equation.

$$\% \text{ recovery of sample (ppm)} = \frac{\text{Recovered Concentration (ppm)}}{\% \text{ Recovery of IS}} \times 100 \%$$

$$\% \text{ of caffeine} = \frac{\text{content of caffeine in sample (mg)}}{\text{mass of sample used (mg)}} \times 100$$

Table 4.1. Mean area of standard calibrator solutions

Conc. (ppm)	Area Under Curve (AUC)			
	1	2	3	Mean
20	1846155	1846652	1846403	1846403
40	3479773	3479999	3479229	3479667
60	5293990	5293717	5294044	5293917
80	6926500	6926697	6926401	6926533
100	8786100	8786469	8786138	8786236
120	10685209	10684015	10686430	10685218

RESULT AND DISCUSSION

No brand of decaffeinated coffee and tea is completely caffeine free; the two standards have been given international standard in which 97% of the caffeine must be removed from the beans or the EU standard in which 99.9% caffeine must be removed from the beans (Blackstock and Colin, 2004). The results indicate that the levels of caffeine obtained by HPLC are within range of

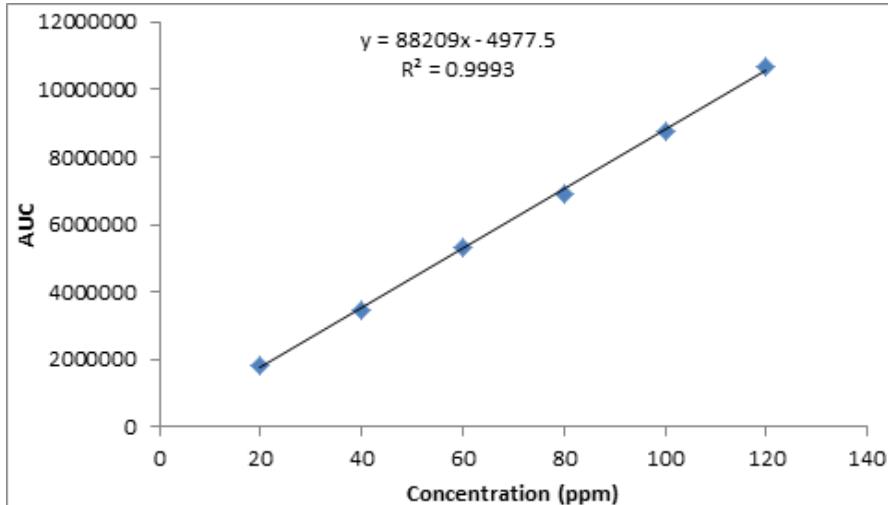


Fig. 4.1. Standard calibration curve of Caffeine

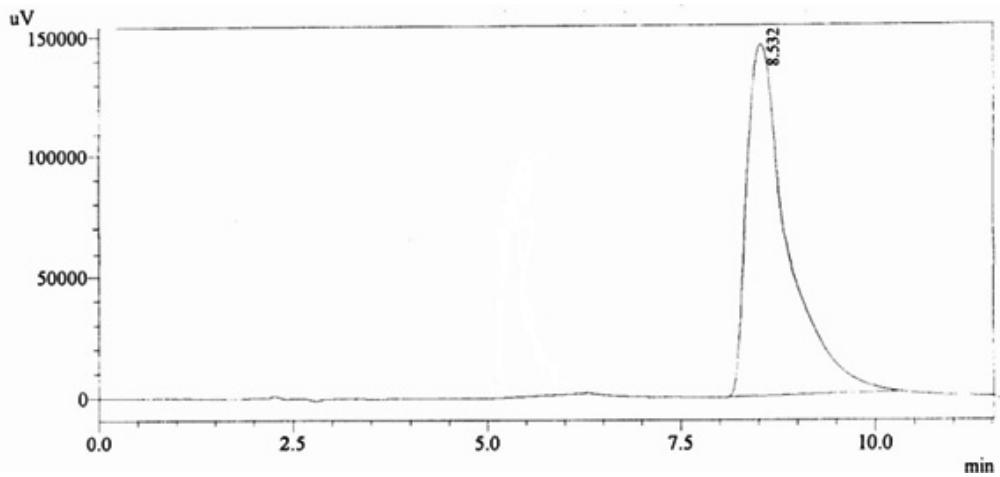


Fig. 4.2. Chromatogram of Retention time of Caffeine

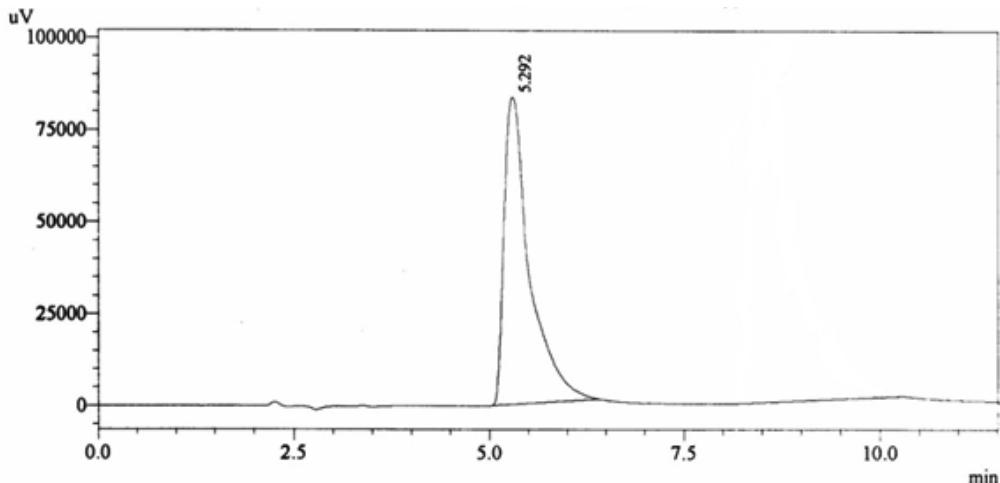


Fig. 4.3. Chromatogram of Retention time of Acetaminophen

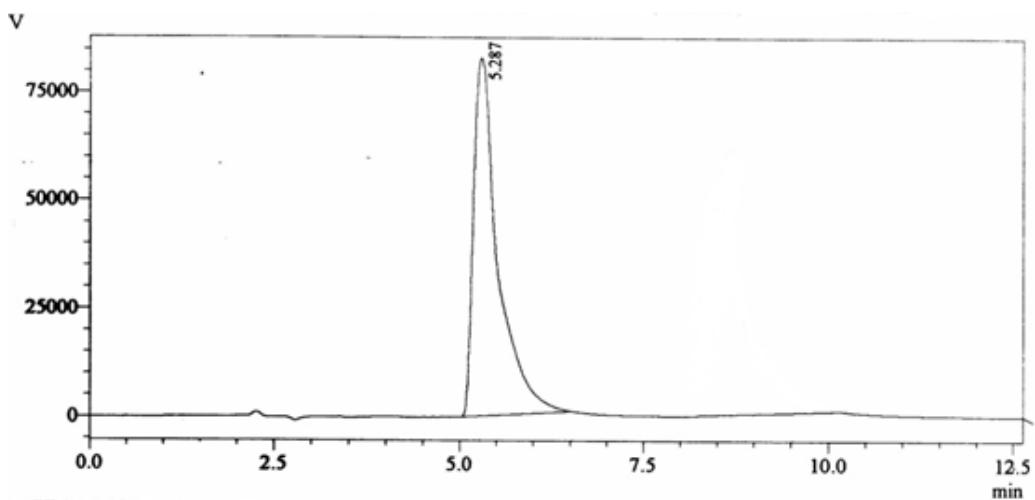


Fig. 4.4. Chromatogram of Negative Quality Control

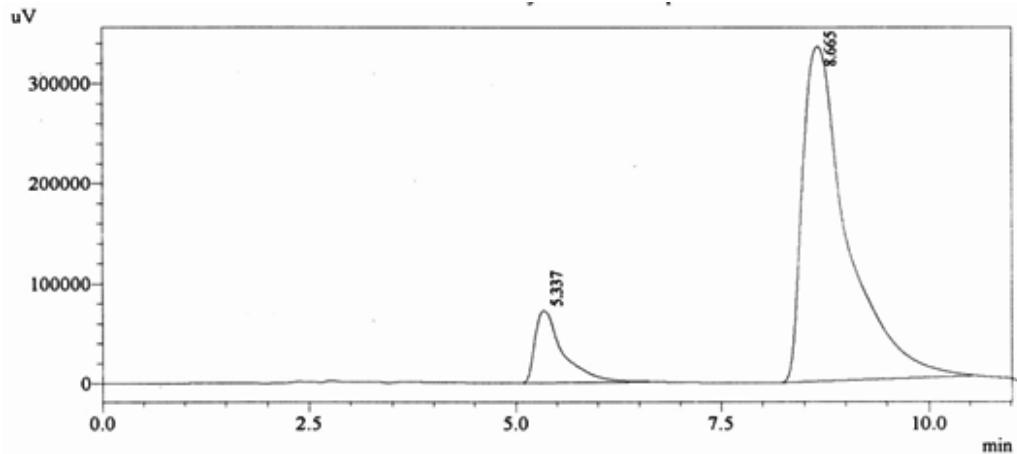


Fig. 4.5. Chromatogram of Positive Quality Control

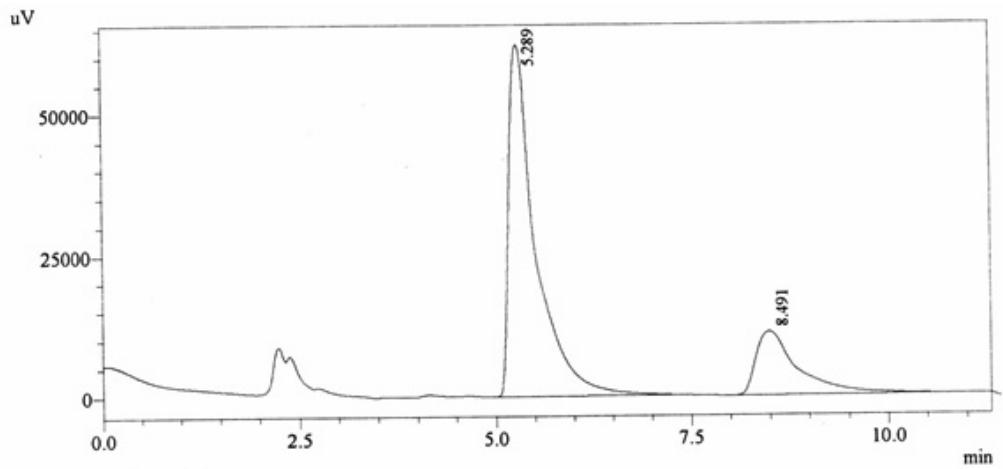


Fig. 4.6. Chromatogram of ABS 1

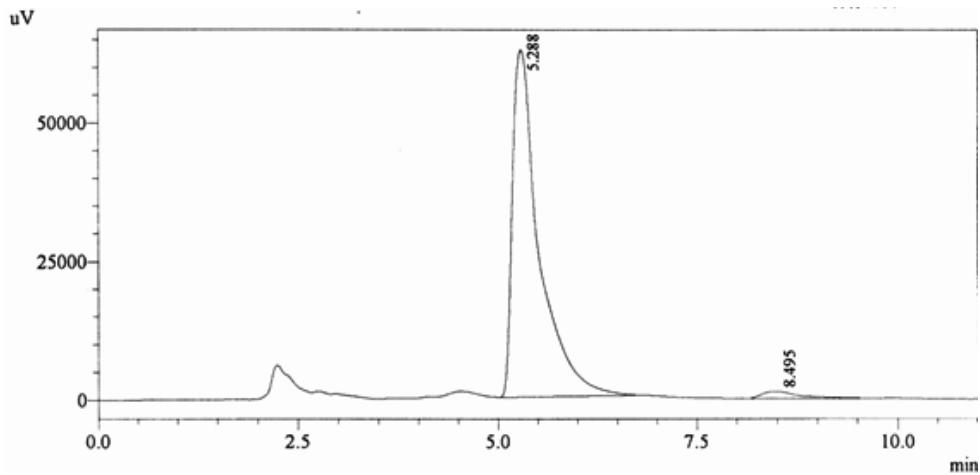


Fig. 4.7. Chromatogram of ABS 2

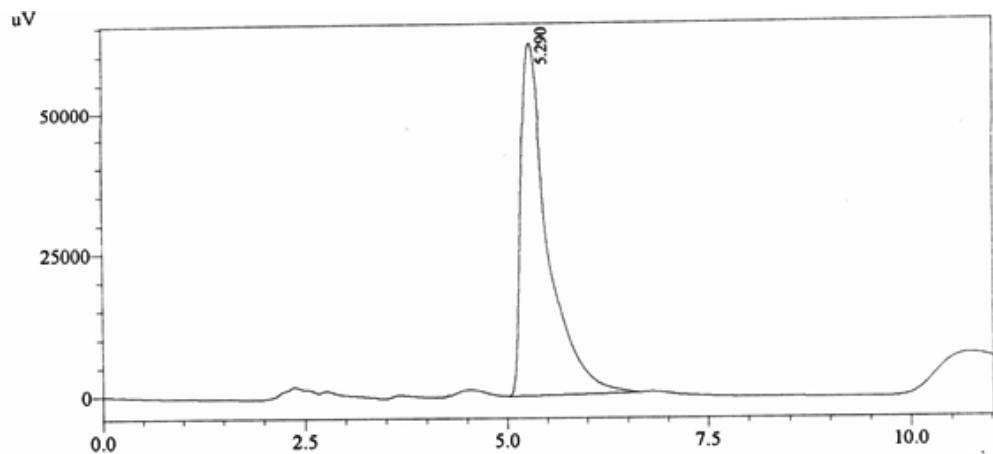


Fig. 4.8. Chromatogram of ABS 3

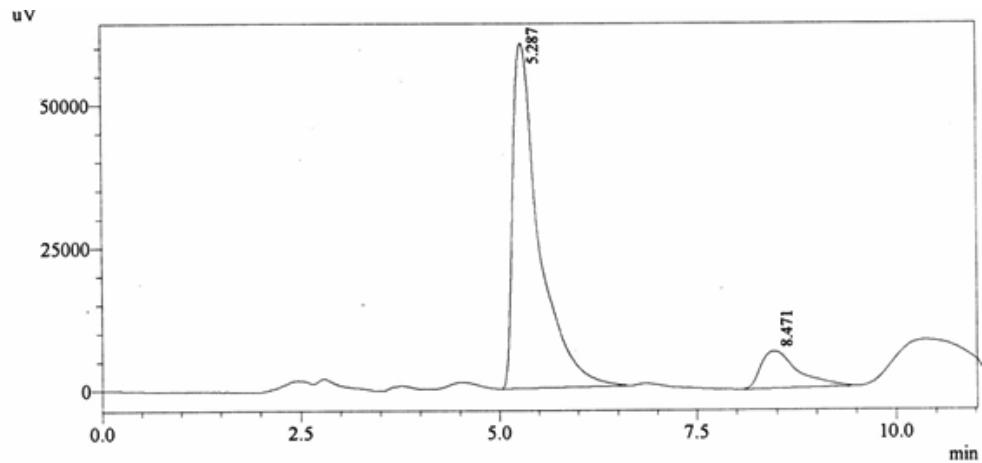


Fig. 4.9. Chromatogram of ABS 4

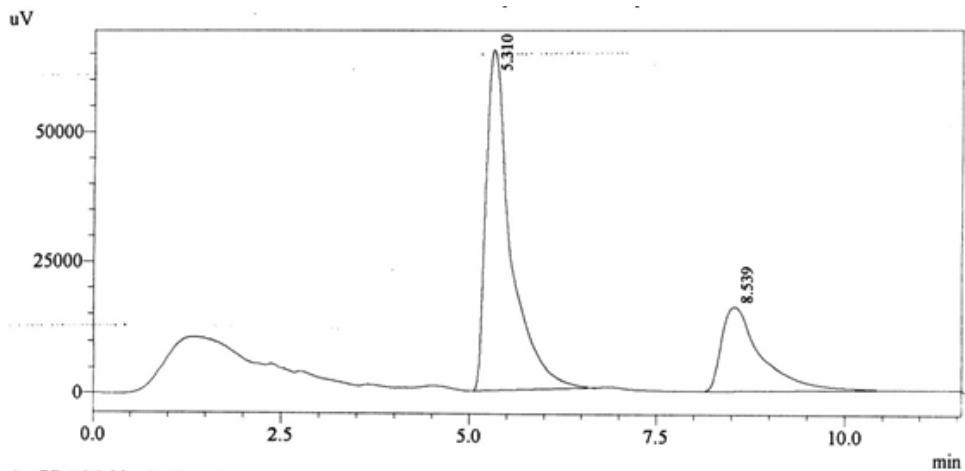


Fig. 4.10. Chromatogram of ABS 5

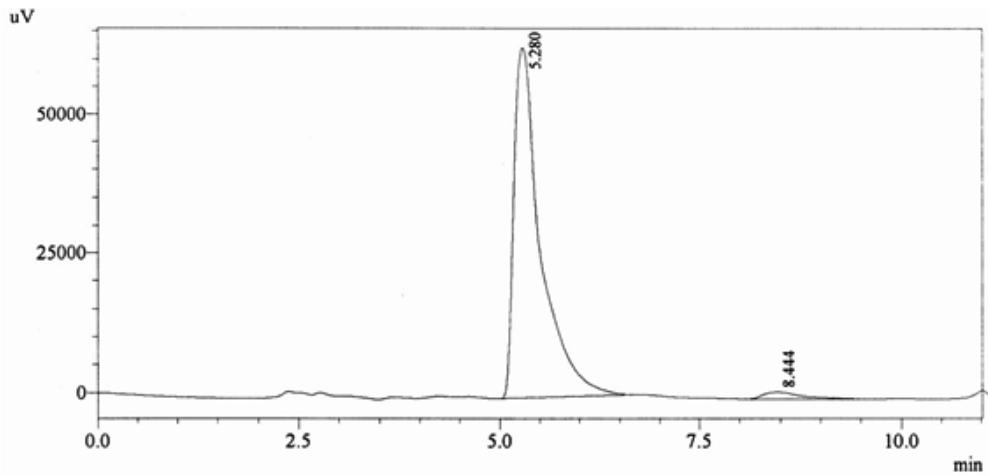


Fig. 4.11. Chromatogram of ABS 6

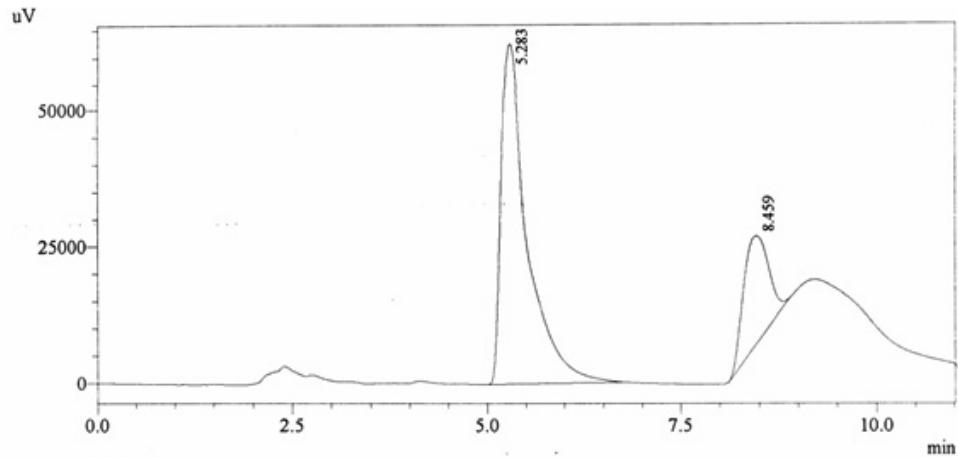


Fig. 4.12. Chromatogram of ABS 7

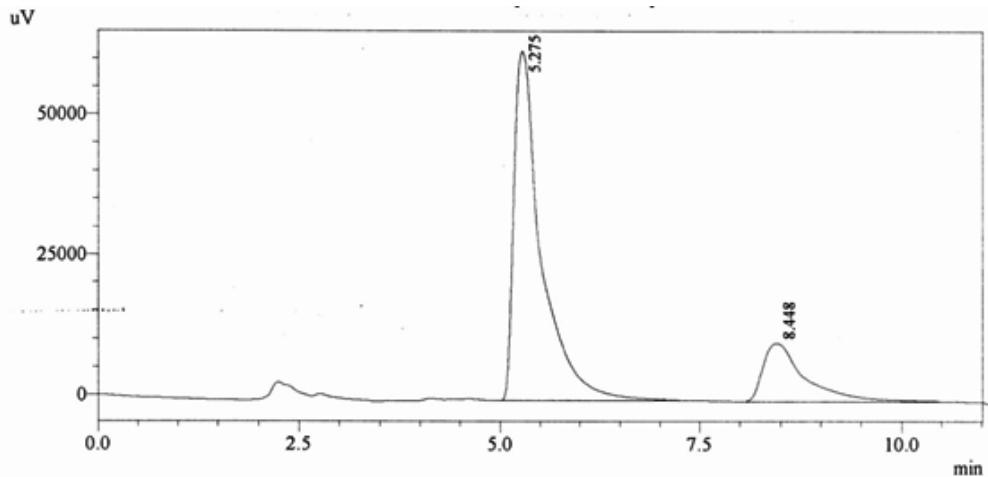


Fig. 4.13. Chromatogram of ABS 8

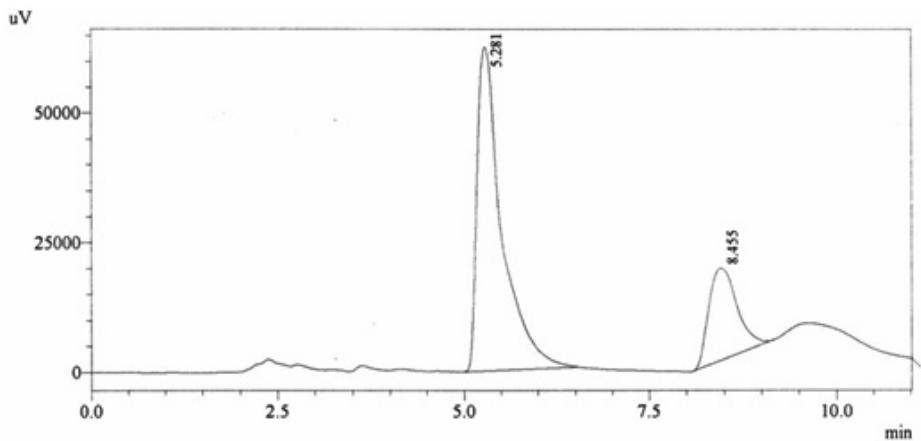


Fig. 4.14. Chromatogram of ABS 9

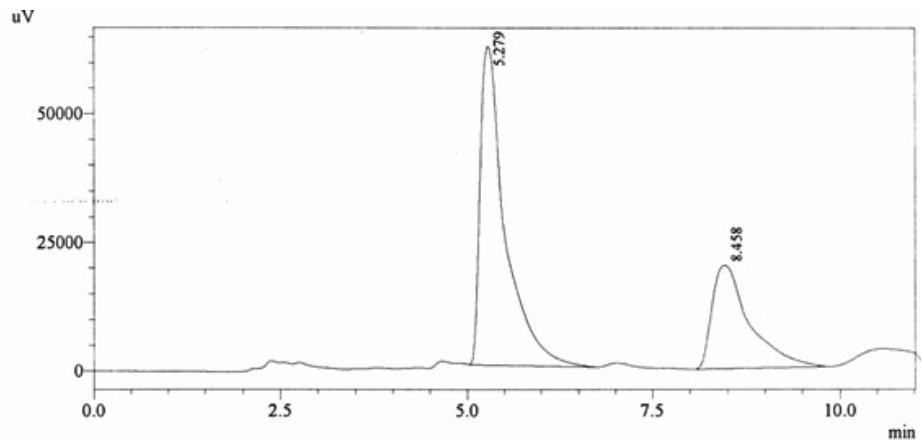


Fig. 4.15. Chromatogram of ABS 10

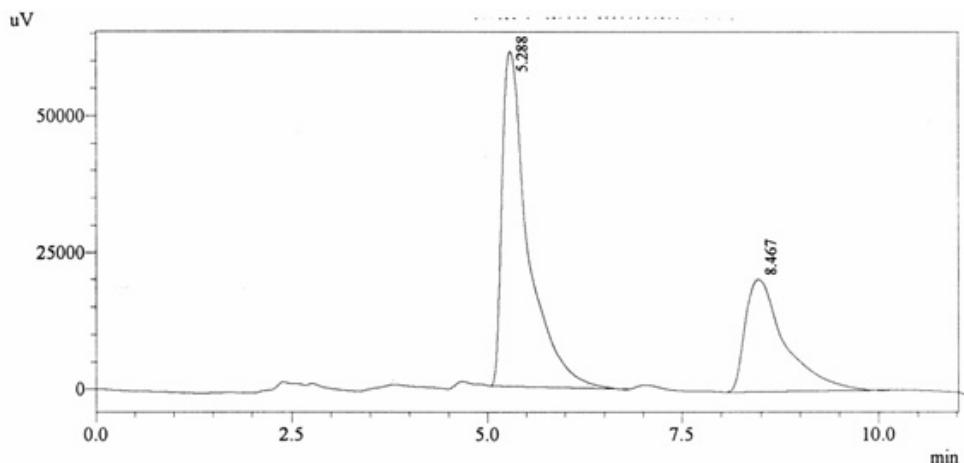


Fig. 4.16. Chromatogram of ABS 11

less than 2.8%, depending on species and origin. The amount of caffeine in coffee and tea depend upon the size of serving, product type and method of preparation (Alves *et al.* 2007).

The calcium hydroxide precipitates the insoluble tannins salts by making the basic solution and it will remove all the interrupting materials that affect the solubility of caffeine. These beverages are prepared by simple water and caffeine can better be estimated by HPLC (Wanyika *et. al* 2010).

Standard Calibration Curve

Standard calibration curves were obtained which indicate a positive linear relationship between the concentration of caffeine standard (ppm) and area of peak (uV) (figure 4.1).

Retention Time Measurement

100 ppm of caffeine solution was run and it gave retention time of 8.532 min (figure 4.2). Further, 40 ppm solution of Acetaminophen (internal standard) produced a peak at 5.292 min showing its retention time (figure 4.3).

Controls

Negative Quality Control

Negative QC gave retention time of 5.287 min and did not show caffeine peak (figure 4.4).

Positive Quality Control

0.2% w/v caffeine solution was run and it gave retention time of 8.665 min and Acetaminophen (internal standard) produced a peak at 5.337 min showing its retention time (figure 4.5).

Samples Profiles

Chromatographic profile of ABS 1

Sample ABS 1 was decaffeinated tea. Its

chromatographic profile shows that AUC of the caffeine is 414079 and that of IS is 1487033 (figure 4.6). This concludes the concentration of caffeine as 0.0041%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 2

Sample ABS 2 was decaffeinated tea. Its chromatographic profile shows that AUC of the caffeine is 42260 and that of IS is 1456377 (figure 4.7). This concludes the concentration of caffeine as 0.0005%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 3

Sample ABS 3 was decaffeinated tea. Its chromatographic profile shows that it does not contain caffeine as no peak of caffeine was detected in chromatogram and area of IS is 1433156 (figure 4.8). Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 4

Sample ABS 4 was decaffeinated tea. Its chromatographic profile shows that AUC of the caffeine is 210353 and that of IS is 1400856 (figure 4.9). This concludes the concentration of caffeine as 0.0022 %. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 5

Sample ABS 5 was decaffeinated tea. Its chromatographic profile shows that AUC of the caffeine is 585993 and that of IS is 1505178 (figure 4.10). This concludes the concentration of caffeine as 0.0057 %. Percentage content was calculated by using equation 2.

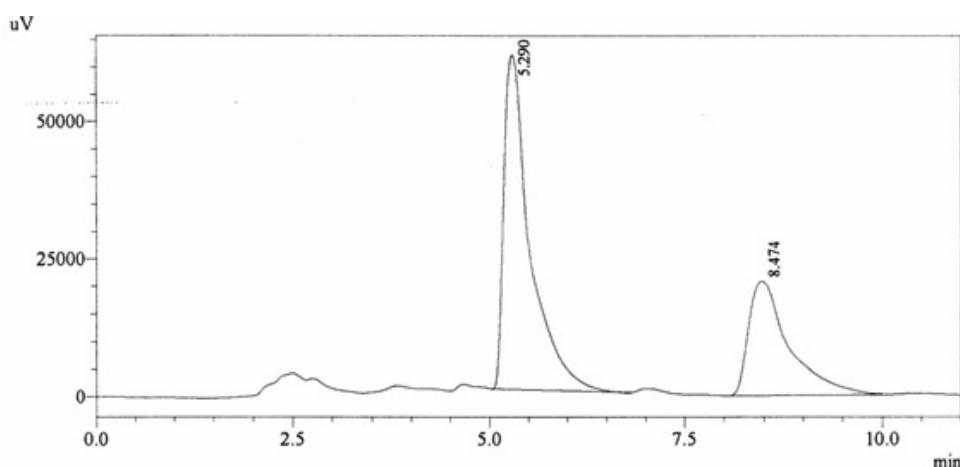


Fig. 4.17. Chromatogram of ABS 12

Chromatographic profile of ABS 6

Sample ABS 6 was decaffeinated tea. Its chromatographic profile shows that AUC of the caffeine is 42677 and that of IS is 1463847 (figure 4.11). This concludes the concentration of caffeine as 0.0005%. Percentage content was calculated by using equation 2

Chromatographic profile of ABS 7

Sample ABS 7 was decaffeinated tea. Its chromatographic profile shows that AUC of the caffeine is 449943 and that of IS is 1463093 (figure 4.12). This concludes the concentration of caffeine as 0.0045 %. Percentage content was calculated by using equation 2

Chromatographic profile of ABS 8

Sample ABS 8 was decaffeinated tea. Its chromatographic profile shows that AUC of the

caffeine is 376163 and that of IS is 1488142 (figure 4.13). This concludes the concentration of caffeine as 0.0037%. Percentage content was calculated by using equation 2

Chromatographic profile of ABS 9

Sample ABS 9 was decaffeinated tea. Its chromatographic profile shows that AUC of the caffeine is 466065 and that of IS is 1453861 (figure 4.14). This concludes the concentration of caffeine as 0.0047%. Percentage content was calculated by using equation 2

Chromatographic profile of ABS 10

Sample ABS 10 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 698053 and that of IS is 1450322 (figure 4.15). This concludes the concentration of caffeine as 0.0070%. Percentage content was calculated by using equation 2.

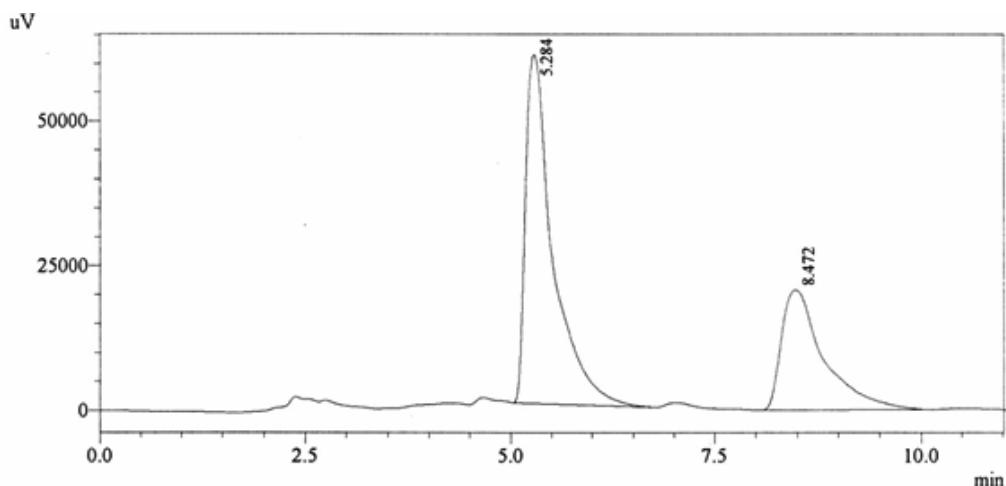


Fig. 4.18. Chromatogram of ABS 13

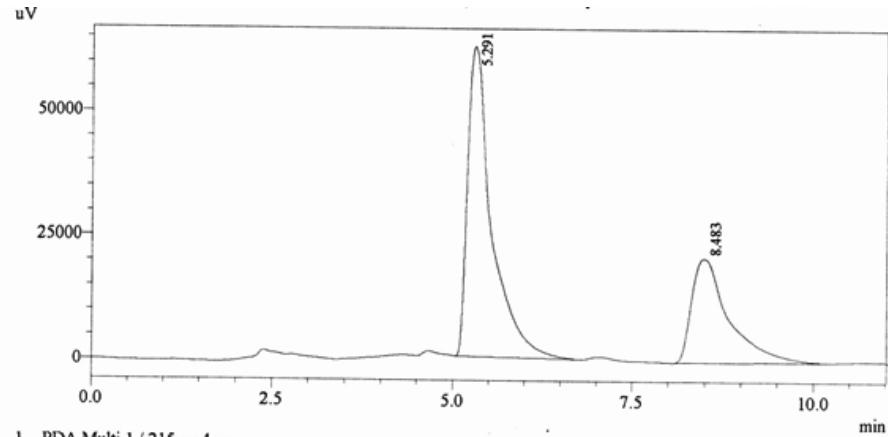


Fig. 4.19. Chromatogram of ABS 14

Chromatographic profile of ABS 11

Sample ABS 11 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 715896 and that of IS is 1430226 (figure 4.16). This concludes the concentration of caffeine as 0.0073%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 12

Sample ABS 12 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 738364 and that of IS is 1418429 (figure 4.17). This concludes the concentration of caffeine as 0.0076%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 13

Sample ABS 13 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 743689 and that of IS is 1408188 (figure

4.18). This concludes the concentration of caffeine as 0.0077%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 14

Sample ABS 14 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 752031 and that of IS is 1456168 (figure 4.19). This concludes the concentration of caffeine as 0.0075%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 15

Sample ABS 15 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 782295 and that of IS is 1452579 (figure 4.20). This concludes the concentration of caffeine as 0.0079%. Percentage content was calculated by using equation 2.

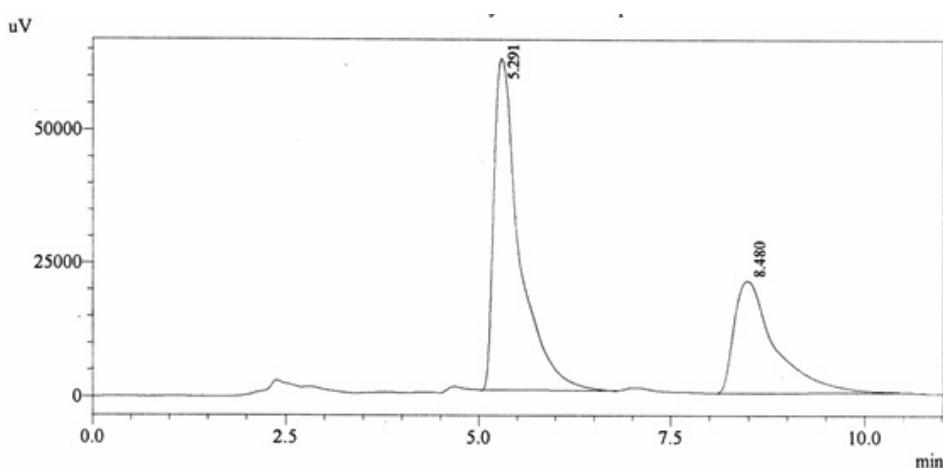


Fig. 4.20. Chromatogram of ABS 15

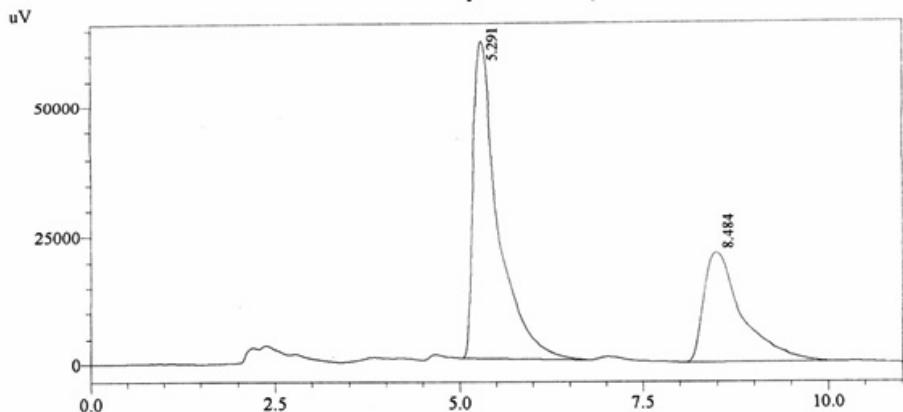


Fig. 4.21. Chromatogram of ABS 16

Chromatographic profile of ABS 16

Sample ABS 16 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 746385 and that of IS is 1439953 (figure 4.21). This concludes the concentration of caffeine as 0.0076%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 17

Sample ABS 17 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 723797 and that of IS is 1438402 (figure 4.22). This concludes the concentration of caffeine as 0.0074%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 18

Sample ABS 18 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 189870 and that of IS is 1448288 (figure

4.23). This concludes the concentration of caffeine as 0.0020%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 19

Sample ABS 19 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 1836744 and that of IS is 1487337 (figure 4.24). This concludes the concentration of caffeine as 0.0180%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 20

Sample ABS 20 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 821681 and that of IS is 1300566 (figure 4.25). This concludes the concentration of caffeine as 0.0092%. Percentage content was calculated by using equation 2.

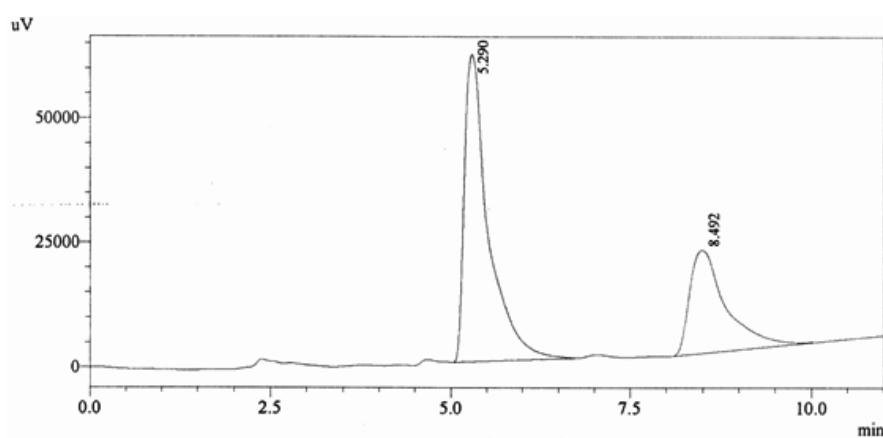


Fig. 4.22. Chromatogram of ABS 17

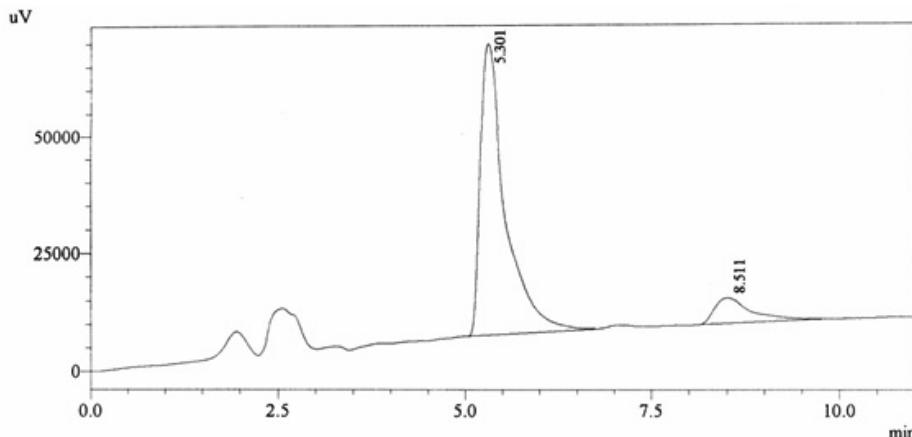


Fig. 4.23. Chromatogram of ABS 18

Chromatographic profile of ABS 21

Sample ABS 21 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 985347 and that of IS is 1413061 (figure 4.26). This concludes the concentration of caffeine as 0.0102%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 22

Sample ABS 22 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 94776 and that of IS is 1485271 (figure 4.27). This concludes the concentration of caffeine as 0.0010%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 23

Sample ABS 23 was decaffeinated coffee. Its chromatographic profile shows that AUC of the

caffeine is 2546 and that of IS is 1534889 (figure 4.28). This concludes the concentration of caffeine as 0.0001%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 24

Sample ABS 24 was decaffeinated coffee. Its chromatographic profile shows that it does not contain caffeine as no peak of caffeine was detected in chromatogram and area of IS is 1473169 (figure 4.29). Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 25

Sample ABS 25 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 1040184 and that of IS is 1200902 (figure 4.30). This concludes the concentration of caffeine as 0.0126%. Percentage content was

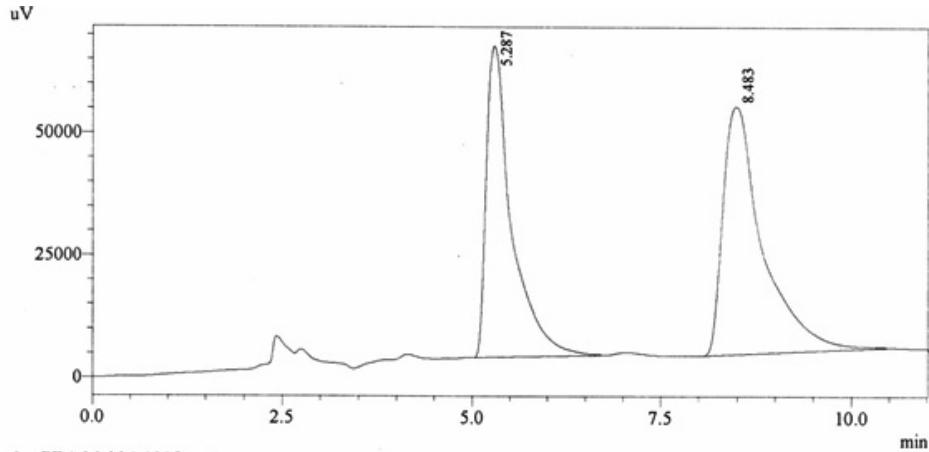


Fig. 4.24. Chromatogram of ABS 19

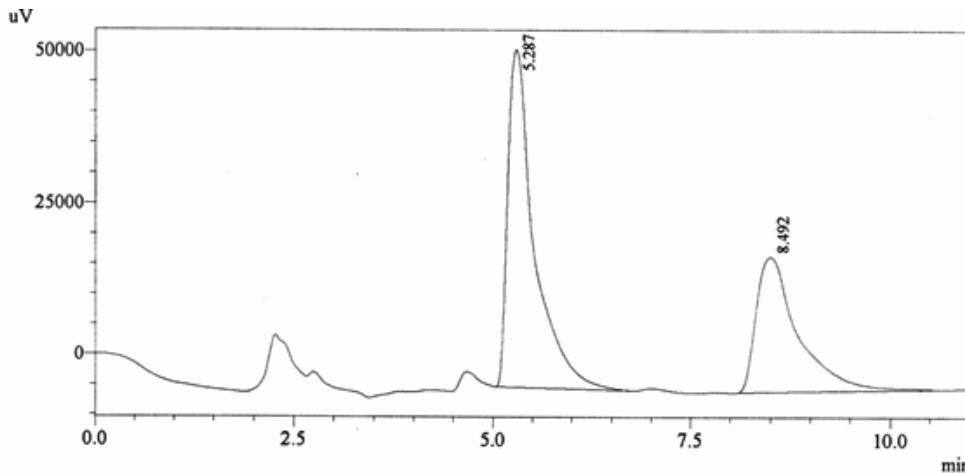


Fig. 4.25. Chromatogram of ABS 20

calculated by using equation 2.

Chromatographic profile of ABS 26

Sample ABS 26 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 749238 and that of IS is 1787173 (figure 4.31). This concludes the concentration of caffeine as 0.0061%. Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 27

Sample ABS 27 was decaffeinated coffee. Its chromatographic profile shows that it does not contain caffeine as no peak of caffeine was detected in chromatogram and area of IS is 1662634 (figure 4.32). Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 28

Sample ABS 28 was decaffeinated coffee. Its chromatographic profile shows that it does not contain caffeine as no peak of caffeine was detected in chromatogram and area of IS is 1733439 (figure 4.33). Percentage content was calculated by using equation 2.

Chromatographic profile of ABS 29

Sample ABS 29 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 1054736 and that of IS is 1693898 (figure 4.34). This concludes the concentration of caffeine as 0.0091%. Percentage content was calculated by using equation 2.

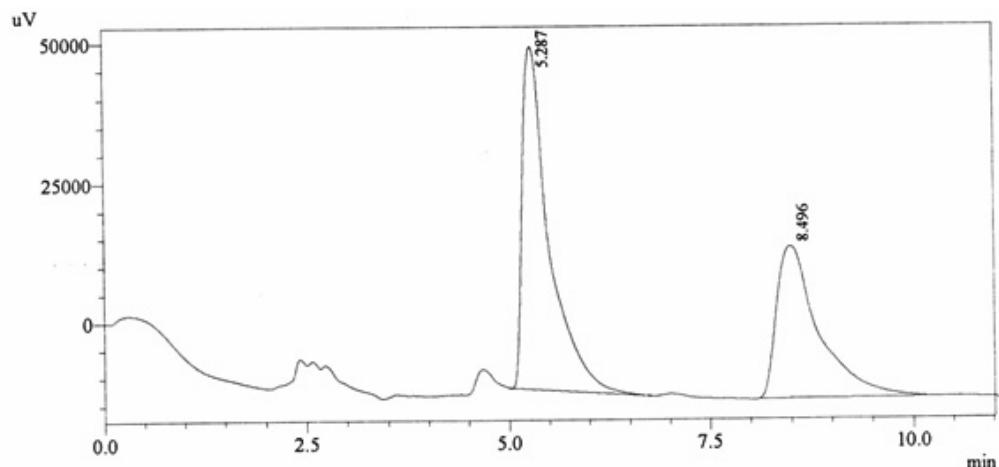


Fig. 4.26. Chromatogram of ABS 21

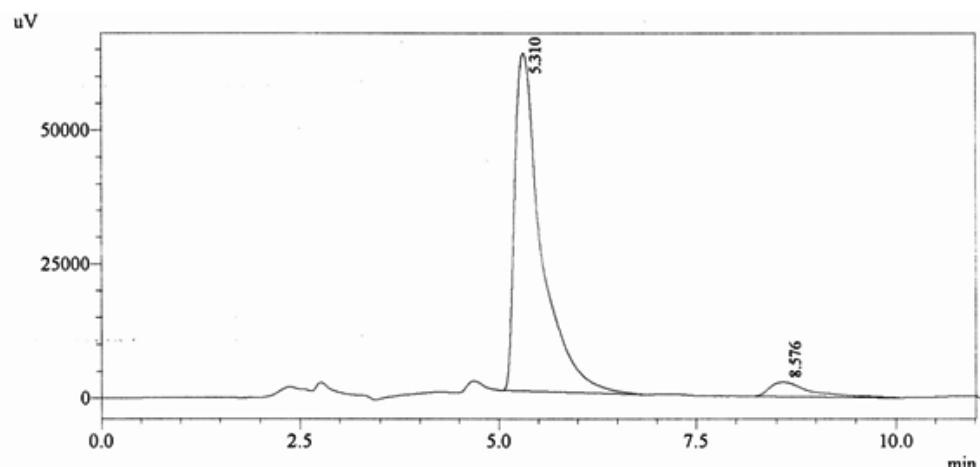


Fig. 4.27. Chromatogram of ABS 22

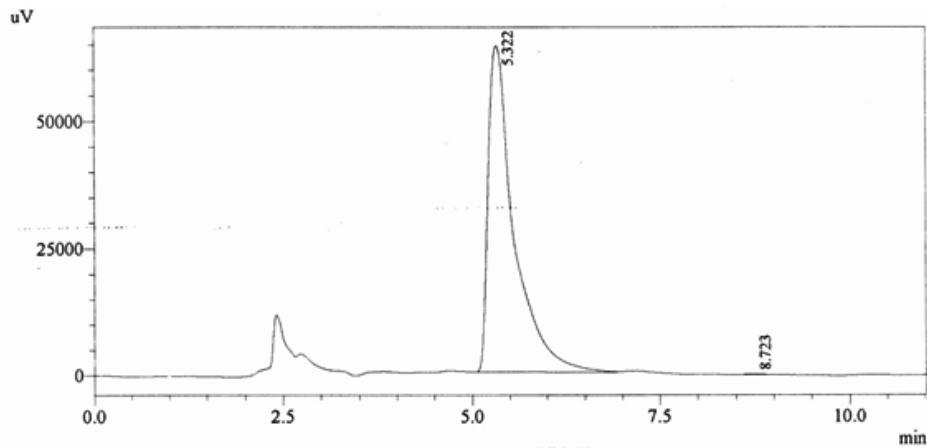


Fig. 4.28. Chromatogram of ABS 23

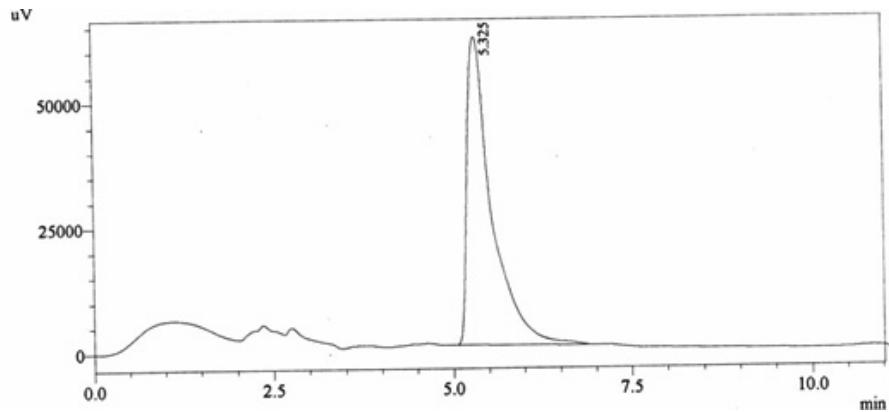


Fig. 4.29. Chromatogram of ABS 24

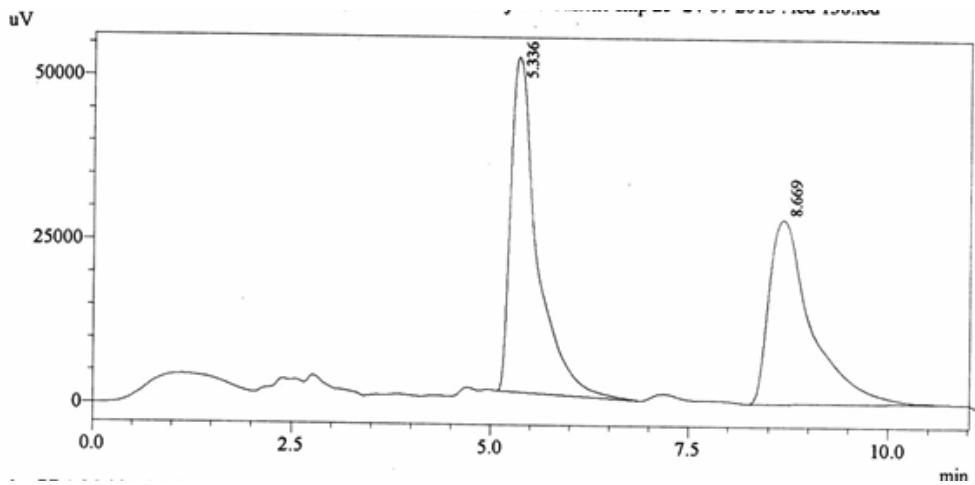


Fig. 4.30. Chromatogram of ABS 25

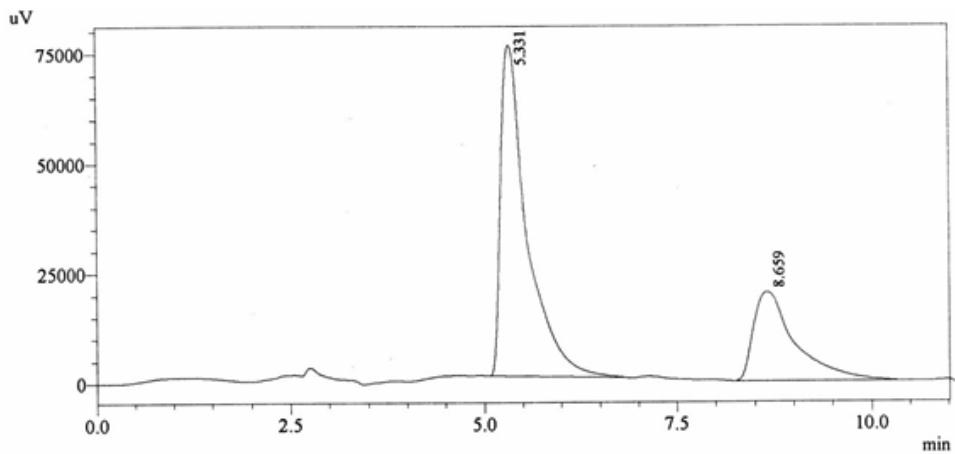


Fig. 4.31. Chromatogram of ABS 26

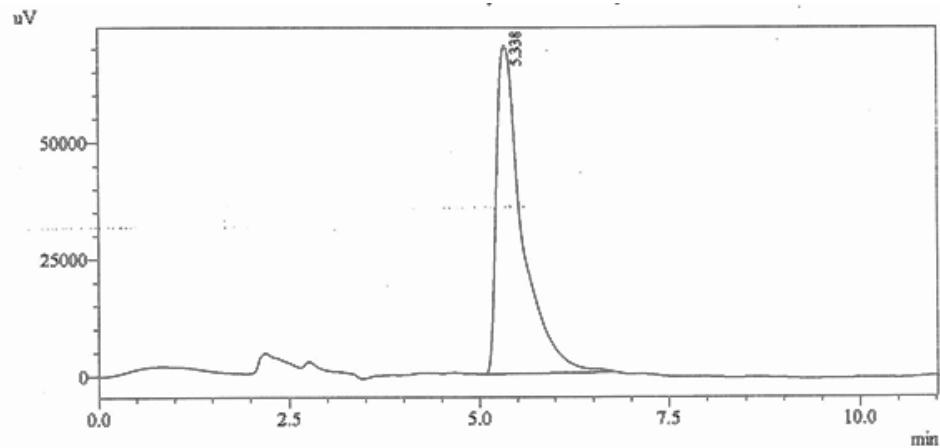


Fig. 4.32. Chromatogram of ABS 27

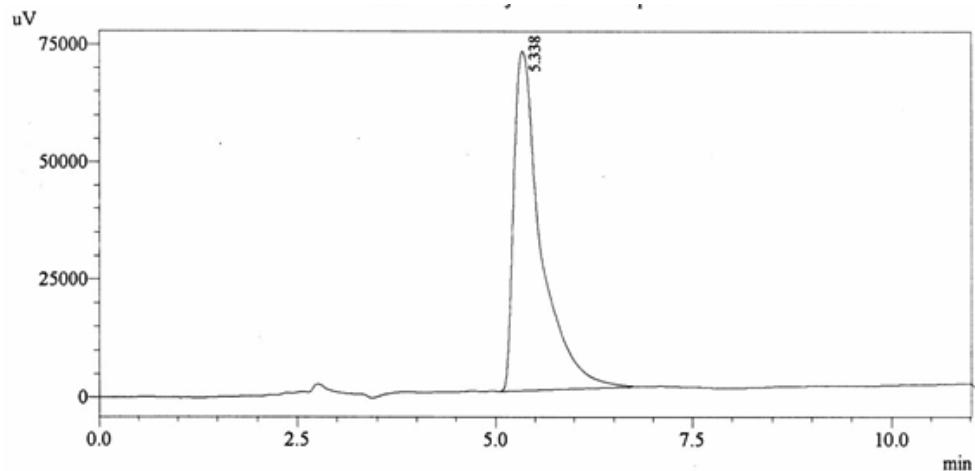


Fig. 4.33. Chromatogram of ABS 28

Chromatographic profile of ABS 30

Sample ABS 30 was decaffeinated coffee. Its chromatographic profile shows that AUC of the caffeine is 1149796 and that of IS is 1692019 (figure 4.35). This concludes the concentration of caffeine as 0.0099%. Percentage content was calculated by using equation 2.

CONCLUSION

No brand of decaffeinated coffee and tea is completely decaffeinated and the two standards have been given international standard in which 97% of the caffeine must be removed from the beans or the EU standard in which 99.9% caffeine must be removed from the beans (Blackstock &

Colin, 2004). 40 ppm (Acetaminophen) and 100 ppm of target analyte (caffeine) were run separately which produced the retention time of 5.292 and 8.532 minutes, respectively. This calibration curve was used to estimate caffeine content. Recovery of internal standard from the calibrators was compared with that of pure IS run and there was no appreciable difference in the recovery. The values of IS in samples were lesser as compared with the response of IS present in the calibrators. This loss of IS in samples was adjusted to purpose the original value. Loss in IS is proportional to the loss of analyte (caffeine) from the sample which can be due to the matrix effects. There are two types of controls that are routinely used as negative and positive controls which are usually found in

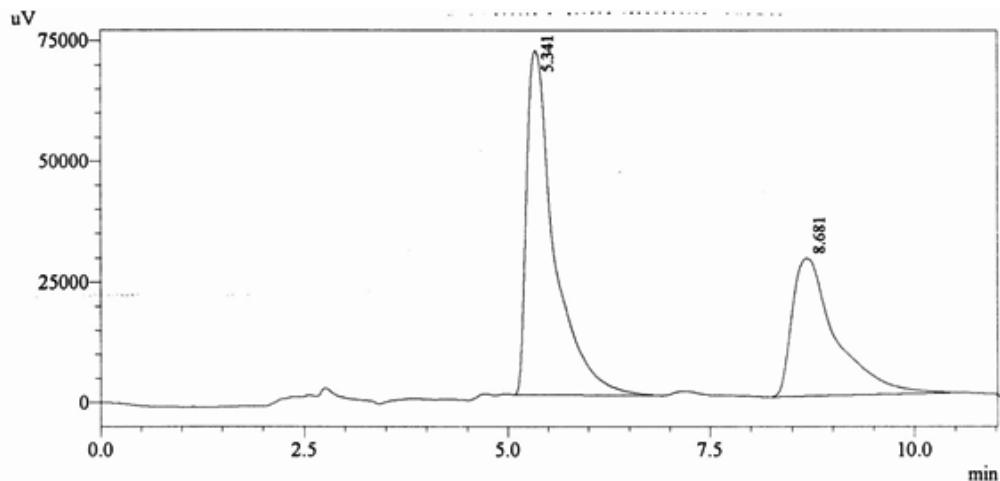


Fig. 4.34. Chromatogram of ABS 29

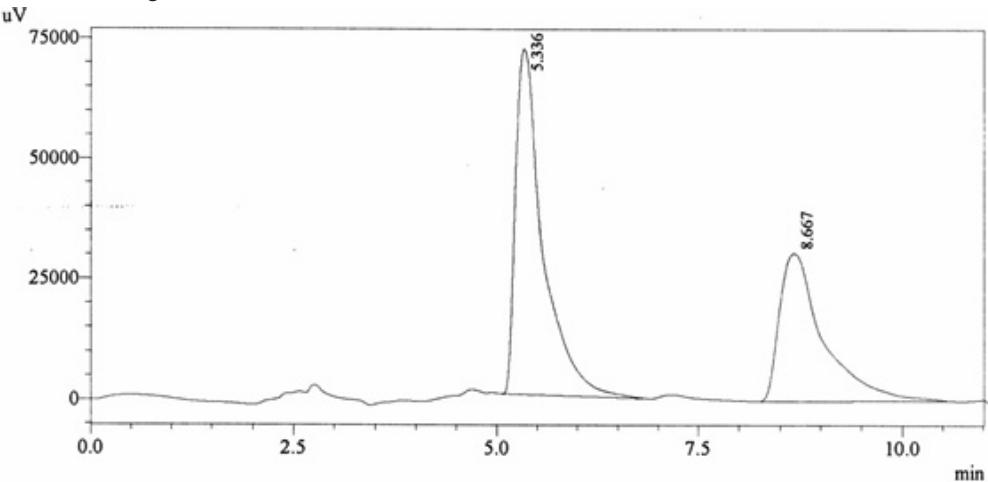


Fig. 4.35. Chromatogram of ABS 30

most type of experiments. The successful results of both controls use to eradicate the error during experiment which means that the expected results will only be positive when the experimental results are positive and expected negative results will indicate that results are negative. The NQC showed no caffeine of target analyte even it was run after the calibrators. This, thus, explains the absence of carry over and provides a mean of reliability of the selected methods. These results are within limits and meet the EU and the international standard for

percentage limits of decaffeinated coffee and tea. According to the international standard 97% of the caffeine should be from the beans and according to the EU standard that 99.9% caffeine-free from beans by mass (6).

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Table 4.2. Calculations of caffeine content:

Sr. No	Sample ID	AUC	Recovered Concentration (ppm)	*Actual Concentration (ppm)	¥ %age of caffeine in sample
1	NQC	0	0	0	0.0000
2	PQC	12634943	143.295	162.62	0.1807
3	ABS 1	414079	4.751	6.13	0.0068
4	ABS 2	42260	0.536	0.71	0.0008
5	ABS 3	0	0.000	0.00	0.0000
6	ABS 4	210353	2.441	3.35	0.0037
7	ABS 5	585993	6.700	8.55	0.0095
8	ABS 6	42677	0.540	0.71	0.0008
9	ABS 7	449943	5.157	6.77	0.0075
10	ABS 8	376163	4.321	5.58	0.0062
11	ABS 9	466065	5.340	7.05	0.0078
12	ABS 10	698053	7.970	10.55	0.0117
13	ABS 11	715896	8.172	10.97	0.0122
14	ABS 12	738364	8.427	11.41	0.0127
15	ABS 13	743689	8.487	11.57	0.0129
16	ABS 14	752031	8.582	11.32	0.0126
17	ABS 15	782295	8.925	11.80	0.0131
18	ABS 16	746385	8.518	11.36	0.0126
19	ABS 17	723797	8.262	11.03	0.0123
20	ABS 18	189870	2.209	2.93	0.0033
21	ABS 19	1836744	20.879	26.96	0.0300
22	ABS 20	821681	9.372	13.84	0.0154
23	ABS 21	985347	11.227	15.26	0.0170
24	ABS 22	94776	1.131	1.46	0.0016
25	ABS 23	2546	0.085	0.11	0.0001
26	ABS 24	0	0.000	0.00	0.0000
27	ABS 25	1040184	11.849	18.95	0.0211
28	ABS 26	749238	8.550	9.19	0.0102
29	ABS 27	0	0.000	0.00	0.0000
30	ABS 28	0	0.000	0.00	0.0000
31	ABS 29	1054736	12.014	13.62	0.0151
32	ABS 30	1149796	13.091	14.86	0.0165

* Calculated by using equation No. 1

¥ Calculated by using equation No. 2

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